Effects of Removal of the Ovary Containing the Largest Follicle on Subsequent Follicular Activity and Function of the Remaining Ovary in Brahman Cows

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Abstract: Effects of removal of the ovary containing the largest follicle (via unilateral ovariectomy) on subsequent follicular activity and ovarian function were evaluated in estrous cycling Brahman cows (n = 9). A sterile bull equipped with a chin-ball marker and daily observations were used for detection of estrus. Ultrasonography to assess ovarian characteristics was performed daily from d 0 (estrus) of the first estrous cycle until the second estrus (time when the ovary with the largest follicle was removed). Before unilateral ovariectomy, 7 out of 9 cows had the CL and the largest follicle on the same ovary (i.e., the active ovary). Following the third observed estrus (i.e., the next estrus occurring after unilateral ovariectomy) the remaining ovary was scanned daily for a complete estrous cycle. Blood samples were collected daily during both scanning periods for analysis of serum concentrations of Progesterone (P_4) by RIA. Cows with one or two ovaries had similar (p>0.10) numbers of follicular waves, estrous cycle lengths, numbers of follicles during the first, second and ovulatory wave and d on which the P_4 peak occurred. However, cows with two ovaries had larger (p<0.003) large-sized follicles (13.5±0.5 mm) and higher (p<0.004) peak serum concentrations of P_4 (10.5±0.9 ng mL^{-1}) than cows with one ovary (10.7±0.5 mm and 7.7±0.9 ng mL^{-1}, respectively). There was a positive correlation between size of the largest follicle and number of ovaries (r = 0.76; p<0.003) and a tendency for a positive correlation between size of the largest follicle and serum peak P_4 concentrations (r = 0.55; p<0.06). In conclusion, removal of the ovary containing the largest follicle did not affect follicular waves or estrous cycle length, but increased follicular populations on the remaining ovary; indicative of ovarian follicular compensatory hypertrophy. In addition, size of the largest follicles and concentrations of P_4 were lower after unilateral ovariectomy.

Key words: Unilateral ovariectomy, ovary, follicle, bovine, progesterone, serum

INTRODUCTION

The observation that unilateral ovariectomy in the pig did not decrease litter size (Hunter, 1787) has led to numerous studies since this observation investigating compensatory changes in the remaining ovary. Staigmiller *et al.* (1974), for example, reported a compensatory hypertrophy of luteal tissue in unilateral ovariectomized pregnant and hysterectomized gilts and this hypertrophy was attributed to an increase in the amount of luteal tissue which, in pregnant gilts, was also dependent on the stage of pregnancy. Similarly, day of the estrous cycle interactions on the presence or absence of ovarian compensatory hypertrophy following unilateral ovariectomy have also been observed (Greenwald, 1962; Hermreck and Greenwald, 1964). The presence of intraovarian (paracrine) interactions between the Corpus Luteum (CL), the dominant follicle and the development of follicular populations (Lazar and Maracek, 1994;

King *et al.*, 1995) supports the concept that intraovarian status may influence ovarian compensatory events and subsequent ovarian function of the remaining ovary following unilateral ovariectomy. The possibility of such interactions are further supported by studies that have shown: That development (i.e., follicular diameter) of the first-wave dominant follicle is greater on the CL-bearing ovary than on the non-CL-bearing ovary (Badinga *et al.*, 1992); that removal of one ovary causes a greater increase in follicular characteristics when a CL is present on the remaining ovary than when it is absent (Mallampati and Casida, 1970) and that the dominant follicle can suppress follicular development in the same ovary, while having a stimulatory effect on the number of medium and large follicles on the ipsilateral ovary (Lazar and Maracek, 1994).

Ovarian follicular compensatory hypertrophy is induced by unilateral ovariectomy in a number of multiple and single ovulating species (Hatai, 1913; Greenwald, 1960; Brinkley *et al.*, 1964; Mallampati and Cassida, 1970;

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Fleming *et al.*, 1984; Christenson *et al.*, 1987; Clutter *et al.*, 1990; Blasco *et al.*, 1994). In cattle, follicular compensatory hypertrophy induced by unilateral ovariectomy has been observed in some studies (Saiduddin *et al.*, 1970; Johnson *et al.*, 1985; Badinga *et al.*, 1992; Lussier *et al.*, 1994; Mohan and Rajamahendran, 1998) and not others (Short *et al.*, 1970). In the ewe it has been demonstrated that total follicular fluid weight and number of follicles (>5 mm in diameter) exhibit compensatory hypertrophy, but only if a CL was present on the ovary compared to CL-removed groups (unilateral ovariectomy or lutectomy), Mallampati and Cassida (1970). Thus, inconsistencies in ovarian follicular compensatory hypertrophy could be associated, as suggested previously, with intraovarian interactions between the CL, presence of a dominant follicle and/or associated follicular populations relative to removal of the more active versus less active ovary.

The objective of this investigation was to evaluate the effects of removal of the ovary containing the largest follicle before ovulation on subsequent estrous cycle length, follicular waves, total follicular populations and serum concentrations of progesterone in relation to unilateral ovariectomy. This study was designed such that each animal served as its own control, with the aim of comparing pre-unilateral ovariectomy estrous cycle characteristics to estrous cycle characteristics subsequent to unilateral ovariectomy.

MATERIALS AND METHODS

Twelve multiparous e s trous cycling Brahman cows having a Body Condition Score (BCS) of 5.2±0.08 (1 = thin, emaciated and 9 = fat, obese; as described by Godfrey *et al.* (1988) were utilized in this investigation. Cows were maintained on Coastal bermudagrass hay which was available *ad libitum* and supplemented with a ration containing corn (1.88 kg/cow/d) and soybean meal (0.32 kg/cow/d). The study described herein followed the General Guidelines for Animal Husbandry as described in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, Savoy, IL).

Cows were maintained with a sterile (i.e., epididymectomized) bull equipped with a chin-ball marker to aid in the detection of estrus. Cows were also observed for estrus behavior twice daily (AM and PM) for at least 30 min at each period. Following observance of estrus behavior (d 0), transrectal ultrasonography (Aloka 210 and a 5 MHz transrectal probe; Aloka Co. Ltd., Wallingford, CT) was performed daily to assess ovarian follicular populations through the majority of the first estrous cycle (Fig. 1). When the largest follicle of the
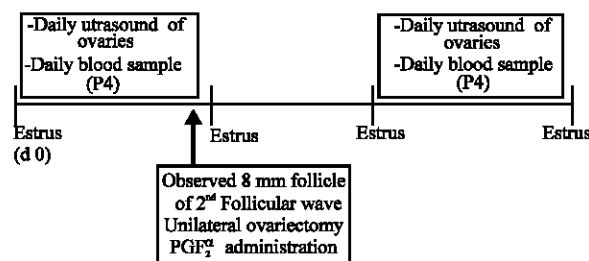

Fig. 1: Experimental Design. Cows were maintained with a sterile bull equipped with a chin-ball marker and observed twice daily throughout the study for the detection of estrus. Following observance of initial estrus behavior (d 0), ultrasonography was performed daily through the first estrous cycle. When the largest follicle of the second follicular wave reached 8 mm in diameter, an intramuscular injection of prostaglandin $F_2\alpha$ was administered and the ovary containing the largest follicle was removed before ovulation by paralumbar laparotomy. After unilateral ovariectomy cows were observed for two additional estrous cycles. At the observance of the third estrus, the remaining ovary was scanned daily for a complete estrous cycle. Blood samples were collected daily during both ultrasound scanning periods for analysis of serum concentrations of progesterone (See Fig. 2).

second follicular wave reached 8 mm in diameter, an intramuscular injection of prostaglandin $F_2\alpha$ ($PGF_2\alpha$; 25 mg, Lutalyse, Pharmacia-Upjohn, Kalamazoo and MI) was given to the cows and the ovary containing the largest follicle was removed before ovulation by paralumbar laparotomy as described by Irvin *et al.* (1978) Fig. 1. It is important to point out that 77.7% of the cows (7 of 9) had the CL and the largest follicle on the same ovary and 22.2% (2 of 9) of the cows did not have the CL and the largest follicle on the same ovary at the time of $PGF_2\alpha$ injection. Therefore, in 77.7% of the cows one could assume that the more active ovary was removed. After unilateral ovariectomy cows were observed for two estrous cycles. At the observance of the third estrus, the remaining ovary was scanned daily for a complete estrous cycle (Fig. 1). Three cows were removed from the experiment due to the absence of, or inconsistencies in, estrus activity (i.e., only 9 of 12 cows completed the study and were utilized in the final analysis).

Each ovary was scanned in more than one plane and at least two images per ovary were recorded using a Sony UP 850 graphics printer (Aloka Co., Ltd., Wallingford, CT). Follicular diameters were measured with a caliper to the

nearest 0.1 mm on the printed images. Measurements were corrected for a 10% distortion in area produced by the printer by Quirk *et al.* (1986). Follicles were classified as small (<4 mm), medium (4.1 to 8 mm) and large (>8.1 mm).

Blood samples were collected daily during both ultrasound scanning periods (Fig. 1). Samples were maintained at 4°C for 24 h, serum harvested and then stored at -20°C until serum concentrations of progesterone were determined by RIA procedures. Serum concentrations of progesterone were determined by a single antibody technique (Williams, 1989). The antibody was #337 anti-progesterone-11-BSA (G.D. Niswender, Colorado State University, Fort Collins, CO). Intra- and inter-assay Coefficients of Variation (CV) were 16 and 23 %, respectively.

Statistical analysis: The variables evaluated were serum concentrations of progesterone, number of follicles during the first, second and ovulatory wave, size of the largest follicle, number of follicular waves, estrous cycle length, peak concentrations of progesterone and day of peak concentration of progesterone. Data were analyzed using SAS GLM for ANOVA and differences between groups were determined by least square means methods using the PDIFF option (SAS, 1985). Correlation coefficients were also calculated for the number of ovaries and size of the largest follicle and size of the largest follicle and peak serum concentrations of progesterone (SAS, 1985).

RESULTS

After unilateral ovariectomy and $PGF_2\alpha$ administration, cows exhibited estrus behavior three to seven days later. Unilateral ovariectomy did not influence (p>0.10) subsequent mean estrous cycle lengths (21.1±0.5 vs. 21.3±0.5 d for pre- and the subsequent estrous cycle post-unilateral ovariectomy, respectively) or mean numbers of follicular waves (2.8±0.2 vs. 2.8±0.2 for pre- and the subsequent estrous cycle post-unilateral ovariectomy, respectively).

Compensatory follicular hypertrophy was observed after the removal of the ovary containing the largest follicle, since there were no differences (p>0.10) between cows with either one or two ovaries in the total number of follicles during the emergence of the first (4.8±0.4 vs. 4.2±0.4), second (4.5±0.4 vs. 4.3±0.4) or ovulatory wave (4.5±0.4 vs. 3.8±0.4; for pre- and the subsequent estrous cycle post-unilateral ovariectomy, respectively). Furthermore, day on which serum concentrations of progesterone peaked was similar (p>0.10) when cows had one (15.6±0.7 d) or two ovaries (16.5±0.7 d). However,
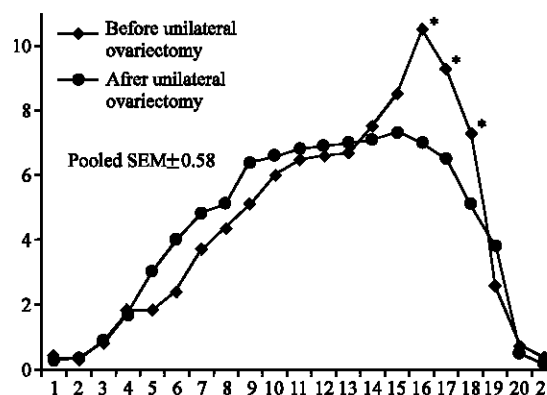

Fig. 2: Serum concentrations of progesterone before and after unilateral ovariectomy in estrous cycling Brahman cows (note: n = 3 cows were removed from analysis due to abnormalities in estrus activity, therefore data represent n = 9 cows). Peak serum concentrations of progesterone differed (p<0.004) between estrous cycles before and after unilateral ovariectomy; as reflected by differences in serum concentration of progesterone noted on days 16, 17 and 18 of the estrous cycle (*, p<0.05)

serum concentrations of progesterone differed (p<0.05) on days 16, 17 and 18 of the estrous cycle (Fig. 2) and peak serum concentrations of progesterone were influenced by the number of ovaries, with cows with two ovaries exhibiting greater (p<0.004) mean peak serum concentrations of progesterone (10.5±0.9 ng mL^{-1}) than when cows had only one ovary (7.7±0.9 ng mL^{-1}; Fig. 2).

Before removal of the ovary containing the largest follicle, cows with two ovaries had larger (p<0.003) large-sized follicles (13.5±0.5 mm) than after unilateral ovariectomy (10.7±0.5 mm). There was also a positive correlation (r = 0.76; p<0.003) between size of the largest follicle and number of ovaries and a tendency for size of the largest follicle and peak serum concentrations of progesterone (r = 0.55; p<0.06) to have a positive correlation; that is, when cows had two ovaries the largest follicle was larger than when cows had only one ovary and cows that had larger follicles had higher serum concentrations of progesterone.

DISCUSSION

Total number of follicles was similar when cows had one or two ovaries, which indicates an increase in follicular numbers of the remaining ovary following unilateral ovariectomy; thus indicating existence of ovarian follicular compensatory hypertrophy in cattle. Similar findings of ovarian follicular

compensatory hypertrophy have been reported previously in cattle (Saiduddin *et al.*, 1970; Johnson *et al.*, 1985; Badinga *et al.*, 1992; Lussier *et al.*, 1994; Mohan and Rajamahendran, 1998) and other species such as the mouse (Clutter *et al.*, 1990), rat (Hatai, 1913), hamster (Greenwald, 1960), rabbit (Fleming *et al.*, 1984; Blasco *et al.*, 1994), sheep (Sundaram and Stob, 1967; Webb *et al.*, 1992) and pig (Brinkley *et al.*, 1964; Redmer *et al.*, 1984; Christenson *et al.*, 1987). It has been suggested that unilateral ovariectomy causes a partial removal of the steroid negative feedback effect, increasing gonadotropin secretion, which stimulates an increase in follicular numbers. While changes in steroidal profiles and associated negative feedback effects on the hypothalamic-pituitary axis are evident (and may also be dependent on the structures on the ovary that were removed; e.g., the CL, dominant follicle, both or other follicular populations), a notable rise in FSH accompanies unilateral ovariectomy stimulating follicular compensatory growth across species see Discussion in Redmer *et al.* (1986) including cattle (Johnson *et al.*, 1985; Badinga *et al.*, 1992) although in a study by Mohan and Rajamahendran (1998) no statistical differences in FSH or LH were noted after unilateral ovariectomy in estrous cycling dry cows and puberal heifers. Nevertheless, in a previous study in heifers, an inhibition of the more typical compensatory rise in FSH prevented the compensatory follicular growth that usually accompanies unilateral ovariectomy (Lussier *et al.*, 1994). The rise in FSH is followed days later by a subsequent rise and then stabilization of inhibin activity (as documented in the gilt), which is implicated in controlling or regulating the degree of follicular growth and ovarian compensatory hypertrophy that occurs after unilateral ovariectomy (Redmer *et al.*, 1986). This has been supported by studies employing the immunization of animals against inhibin, which has further implicated inhibin as a local mediator of ovarian responsiveness to unilateral ovariectomy and the subsequent compensatory follicular hypertrophy that ensues (King *et al.*, 1993; 1995).

Studies in cattle (Echternkamp and Gregory, 2002) and some other species (human: Fukada *et al.*, 2000) agree that the right ovary (55%) is more active than the left ovary (45%). When removing the ovary with the largest follicle before ovulation and if it contains the CL as did 77.7% of cows in this study, the more active ovary may be removed with the remaining ovary being, in a relative sense, somewhat less active. In this study, the large follicles of the estrous cycle subsequent to unilateral ovariectomy were smaller and peak serum concentrations of progesterone were lower than those of the estrous cycle pre-unilateral ovariectomy. Similarly, Mohan and

Rajamahendran (1998) found that the largest ovulatory follicle in the remaining ovary after unilateral ovariectomy was smaller in diameter than its counterpart during the control cycle before unilateral ovariectomy. However, they also noted no significant effect of unilateral ovariectomy on plasma concentrations of progesterone in cows or heifers (Mohan and Rajamahendran, 1998), which is in contrast to the present study. Obviously, intrafollicular factors, such as inhibin, activin, IGFs, etc., play an important role in follicular and luteal growth, differentiation and function and their expression/secretion is likely dependent on intraovarian status (e.g., follicular populations or presence of a CL). The positive correlations found in this study between the number of ovaries (one or two) and size of the large follicles and between size of the large follicles and progesterone secretion are suggestive of the influence of possibly removing the more active versus less active ovary on subsequent follicular compensatory hypertrophy events. Lazar and Maracek (1994) reported that the presence of the CL had a negative effect at any stage of the estrous cycle on the recruitment of small follicles when located in the same ovary. In addition, in this same study, the presence of a dominant follicle inhibited follicular development in the same ovary, but had a positive effect on the number of medium- and large-sized follicles on the ipsilateral ovary. More specifically, Mallampati and Casida (1970) found in the ewe that removal of one ovary caused a greater increase in follicular characteristics (follicular fluid weight and number of follicles >5 mm in diameter) when a CL was present in the remaining ovary than when it was absent. While the subtle differences in peak progesterone and large follicle sizes were noted, the physiological relevance of these remains unclear as other aspects of the estrous cycle subsequent to unilateral ovariectomy were not affected (e.g., estrous cycle length and number of follicular waves). Similarly, Mohan and Rajamahendran (1998) found no alterations in estrous cycle length or number of follicular waves in estrous cycling dry cows and puberal heifers when comparing estrous cycles before and after unilateral ovariectomy.

CONCLUSION

In summary, removal of the ovary with the largest follicle before ovulation had no effect on subsequent follicular waves or estrous cycle length in relation to pre-unilateral ovariectomy status; indicating induction of follicular compensatory hypertrophy of the remaining ovary. However, size of the largest follicles and peak circulating concentrations of progesterone were lower for the remaining ovary in a subsequent estrous cycle

following unilateral ovariectomy. We suggest a possible association between removal of the more active ovary and observations of smaller dominant follicles and reduced peak serum concentrations of progesterone from the remaining ovary in subsequent estrous cycles; although this requires further study to substantiate.

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